ALLELES OF THE HUMAN MU OPIOID RECEPTOR, DIAGNOSTIC METHODS USING SAID ALLELES, AND METHODS OF TREATMENT BASED THEREON

CROSS-REFERENCE TO RELATED APPLICATION

This Application claims priority to provisional application Serial No. 60/212,225, filed June 16, 2000, incorporated herein by reference in its entirety.

GOVERNMENTAL SUPPORT

This invention was made government support under Grant Nos. NIH-NIDA P50-DA05130 and NIH-NIDA K05-DA00049 awarded by the National Institute of Drug Addiction. The Government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to alleles of the human mu opioid receptor gene, along with products derived from such alleles. Also included herein are methods of diagnosing various susceptibilities using such alleles and determining treatment for certain diseases based upon the presence of specific alleles of the human mu opioid receptor gene, and various diseases or disorders related to physiological functions regulated by the hypothalamus pituitary adrenal axis (HPA) or the hypothalamus pituitary gonadal axis (HPG).

BACKGROUND OF THE INVENTION

Opioid drugs have various effects on perception of pain, consciousness, motor control, mood, autonomic function, and can also induce physical dependence. The endogenous opioid system plays an important role in modulating endocrine, cardiovascular, respiratory, gastrointestinal functions, and immune functions. Opioids, either exogenous or endogenous, exert their actions by binding to specific membrane-associated receptors.

Examples of exogenous opioids presently known include, opium, heroin, morphine, codeine, fentanyl, and methadone, to name only a few. Moreover, a family of over 20 endogenous opioid peptides has been identified, wherein the members possess common structural features, including a positive charge juxtaposed with an aromatic ring that is required for interaction

with an opioid receptor. It has been determined that most, if not all the endogenous opioid peptides are derived from the proteolytic processing of three precursor proteins, i.e., pro-opiomelanocortin, proenkephalin, and prodynorphin. In addition, a fourth class of endogenous opioids, the endorphins, has been identified (the gene encoding these proteins has not yet been cloned). In the processing of the endogenous opioid precursor proteins, initial cleavages are made by membrane-bound proteases that cut next to pairs of positively charged amino acid residues, and then trimming reactions produce the final endogenous opioids secreted from cells *in vivo*. Different cell types contain different processing enzymes so that, for example proopiomelanocortin can be processed into different endogenous peptides by different cells. For example, in the anterior lobe of the pituitary gland, only corticotropin (ACTH), β-lipotropin, and β-endorphin are produced. Both pro-enkephalin and pro-dynorphin are similarly processed by specific enzymes in specific cells to yield multiple opioid peptides.

Pharmacological studies have suggested there are numerous classes of opioid receptors which bind to exogenous and endogenous opioids. These classes differ in their affinity for various opioid ligands and in their cellular and organ distribution. Moreover, although the different classes are believed to serve different physiological functions, there is substantial overlap of function, as well as of distribution.

In particular, there are at least three known types of opioid receptors, mu (μ), delta (δ), and kappa (κ), to which morphine, the enkephalins, and the dynorphins can bind. These three opioid receptor types are the sites of action of opioid ligands producing analgesic effects. However, the type of pain inhibited and the secondary functions vary with each receptor type. The mu receptor is generally regarded as primarily associated with pain relief, and drug or other chemical dependence, i.e., addiction and alcoholism.

The human mu opioid receptor, which modulates corticotropin releasing hormone, has been isolated and described in PCT Application WO 95/07983 (March 23, 1995) (SEQ ID NO:1) as well as in Chen, Y., Mestek, A., Hurley, J.A., & Yu, L. (1993) *Mol. Pharmacol.* 44, 8-12, and Wang, et al., *FEBS Letters*, (1994)338:217-222. Furthermore, SEQ ID NO:1 can readily be obtained in GENBANK under accession number L25119. The cDNA therefor contains an

open reading frame capable of encoding a protein of 400 amino acid residues with 94 % sequence similarity to the rat mu opioid receptor. Hydropathy analysis of the deduced protein indicates the presence of seven hydrophobic domains, typical of G-protein-coupled receptors. The N-terminus contains five potential N-linked glycosylation sites which remain conserved between the human and the rat mu opioid receptor. A variant in which Asn-40 is changed to Asp (N40D) is reported in GENBANK Accession No. U12569. New polymorphisms G24A (silent), G779A (Arg260His), and G942A (silent) of the mu opioid receptor have been described in co-pending application Serial No. 09/113,426, filed July 10, 1998, and Serial No. 09/351,198, filed July 9, 1999, both of which are incorporated herein by reference in their entireties.

In the body and brain, heroin is biotransformed to morphine, which acts at the mu opioid receptor and results in an euphoric effect and confers the reinforcing properties of the drug and contributes to development of addiction. Heroin addiction can be managed through treatment, primarily methadone maintenance. However, the biological basis of heroin addiction may include diversity of gene structure. Such genetic diversity of the human mu opioid receptor, and the impact of such diversity on receptor function, could contribute to the success or failure of pharmacological management. Similar problems with respect to patient response to pharmacological treatment could occur in most, if not all addictive diseases, such as heroin addiction, alcohol addiction, or cocaine addiction to name only a few, or a combination thereof.

Moreover, addiction to opioid drugs, especially heroin, is a major social problem in the United States, and throughout the world. For example, recent epidemiological assessments sponsored by the NIH-NIDA and other federal agencies have found that around 2.7 million persons in the United States have used heroin at some time. Moreover, the numbers of "hardcore" long-term heroin addicts (addiction being defined herein as self administration of a regular, multiple, daily dose use of a short-acting opioid, such as heroin, for one year or more, with the development of tolerance, physical dependence and drug-seeking behavior, a definition codified in the Federal guidelines governing pharmacotherapy using long-acting agents such as methadone or LAAM, and used as the minimal requirement for entry into treatment) are now

estimated to be approximately one million persons. In addition, it has been estimated that around 24 million persons in the United States have used cocaine for some time, and of that number, approximately one million use cocaine regularly, and at least 600,000-700,000 are cocaine addicts.

In view of the importance of the human mu opioid receptor in the study of addiction, and the epidemic proportions of drug addiction, especially to heroin, alcohol or cocaine, or a combination thereof, in the United States and throughout the world, and its involvement in the neuroendocrine system, and physiological functions regulated thereby, efforts have been made to investigate whether any polymorphisms in the gene encoding the human mu opioid receptor exist in the population, and whether such polymorphisms result in a phenotype that has an increased or decreased susceptibility towards development of addiction to exogenous opioids, such as heroin, or alcohol, cocaine, or other addictive drugs. For example, in an article entitled "Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse" (Berrettini, W.H., Hoehe, M.R., Ferraro, T.N., DeMaria, P.A., and Gottheil, E., *Addiction Biology* **2**:303-308 (1997)), two polymorphisms in the human mu opioid receptor gene were reported. One polymorphism (G to T) occurs at nucleotide 175 preceding initiation of translation, and a second coding polymorphism C to T) at nucleotide 229 (with respect to transcription initiation) on exon I results in an Ala to Val residue change. However, data taken from a study indicated the C229T polymorphism does not differ in occurrence with statistical significance in addicts relative to non addicts (*idem* at 306). No functional studies were reported.

It has been further determined that a receptor for both endogenous and exogenous opioids modulates the activity of the hypothalamus pituitary adrenal axis (HPA) and the hypothalamus pituitary gonadal axis (HPG), which effects the neuroendocrine system and its production of signaling compounds that play important roles in regulation of numerous physiological functions. In particular, the neuroendocrine system involves the integration of the neural and endocrine systems of the body, and is responsible for the coordination of numerous bodily functions. An important part of this system is the hypothalamus, a specialized portion of the brain involved in receiving and relaying messages from the central nervous system to other

parts of the body. Upon stimulation by chemical signals from the central nervous system, the hypothalamus secretes hypothalamic hormones, such as corticotropin releasing factor (CRF) or hormone and gonadotropin releasing hormone or luteinizing hormone releasing hormone. These factors in turn stimulate the anterior pituitary gland to secrete tropic hormones, or tropins, which are synthesized as relatively long polypeptides, and then are then biotransformed to produce active peptide hormones. Pro-opiomelanocortin, which is processed into several active peptide hormones, including adrenocorticotropic hormone (ACTH), is an example of a tropic hormone. ACTH stimulates the adrenal cortex to secrete additional hormones, like cortisol, a stress hormone in humans which regulates glucose metabolism, and targets many tissues in the body. In addition, examples of hormones produced by the anterior pituitary glad upon stimulation with gonadotropin releasing hormone include follicle-stimulating hormone and luteinizing hormones. These hormones stimulate the gonads, such as the ovaries and the testes, to secrete androgens, such as testosterone, progesterone, and estrogen, which in turn affect sexual development, sexual behavior, and other reproductive and nonreproductive functions. As a result, the endogenous opioid system plays an important role in modulating endocrine, reproductive, cardiovascular, respiratory, gastrointestinal, immune functions, sexual development and function, as well as a person's response to stress.

More specifically, in humans, it has been determined that chronic administration of opioids has an inhibitory effect on the HPA axis [McDonald *et al.*, Effect of morphine and nalorphine on plasma hydrocortisone levels in man. *J. Pharmacol. Exp. Ther.* **125**:241247 (1959)]. Basal levels of ACTH and cortisol are significantly disrupted in active heroin addicts: suppression of ACTH and cortisol and abnormal diurnal rhythms of these hormones are found [Kreek, Medical safety and side effects of methadone in tolerant individuals. *JAMA* **223**:665-668 (1973)]. Basal levels and the diurnal rhythm of ACTH and cortisol, which are disrupted in active heroin addicts, have been shown to become normalized in moderate to high dose, long-term methadone-maintained patients when compared to those of healthy volunteer subjects [Kreek, 1973; Kreek *et al.*, Circadian rhythms and levels of beta-endorphin, ACTH, and cortisol during chronic methadone maintenance treatment in humans. *Life Sci.* **33**:409-411 (1983); Kreek *et al.*, Prolonged (24 hour) infusion of the opioid antagonist naloxone does not significantly alter plasma levels of cortisol and ACTH in humans. *Proceedings of the 7th*

International Congress on Endocrinology, Elsevier Science, p. 1170, 1984].

In healthy volunteers, ACTH and cortisol levels decrease below the basal levels in response to the infusion of β-endorphin indicating feedback of inhibition of pituitary ACTH release or suppression of hypothalamic CRF release by β-endorphin [Taylor *et al.,* Beta-endorphin suppresses adrenocroticotropin and cortisol levels in normal human subjects. *J. Clin. Endocrinol. Metab.* **57**:592-596 (1983)], and also naloxone (an opioid antagonist) stimulates a rise in serum ACTH and cortisol, suggesting that the HPA axis is under the tonic inhibitory control of endogenous opioids normalized in steady-state chronic methadone-maintained patients; their HPA axis responses to metyrapone-induced stress appear to be no different from that of healthy volunteer subjects [Kreek, 1973; Kreek *et al., Prolonged (24 hour) infusion of the opioid antagonist naloxone does not significantly alter plasma levels of cortisol and ACTH in humans. Proceedings of the 7th International Congress on Endocrinology* Elsevier Science, p. 1170, 1984].

Support for the effects of opioids on physiological functions regulated by the HPA and the HPG axes can be found in observations of heroin addicts. More specifically, it has been observed that many heroin addicts are infertile, and in the case of female addicts, their menstrual cycle is dramatically disrupted to the point that they do not ovulate. Furthermore, it has been observed that heroin addicts, and nonaddicted patients taking morphine, become constipated, and that the immune systems of addicts is weakened relative to the immune system of non addicts. However, once therapeutic agents designed to treat addiction, such as methadone, addicts become fertile, are no longer constipated, and have a immune system whose ability to fight foreign bodies is in parity with the immune system of a nonaddict.

Hence, what is needed is discovery of additional, heretofore unknown polymorphisms of the human mu opioid receptor gene that can be used as genetic markers to map the locus of the human mu opioid receptor gene in the genome.

What is also needed are the DNA sequences of heretofore unknown isolated nucleic acid molecules which encode human mu opioid receptors, wherein the DNA sequences include a

combination of presently known and subsequently discovered polymorphisms of the human mu opioid receptors.

Furthermore, what is needed is the characterization of the binding properties of heretofore unknown human mu opioid receptors produced from the expression of genes comprising such heretofore unknown polymorphisms of the human mu opioid receptor gene, or combinations of unknown polymorphisms and known polymorphisms.

Furthermore, what is needed is a characterization of the activity of such unknown human mu opioid receptors produced from the expression of nucleic acid molecules comprising such polymorphisms.

What is also needed is a correlation between polymorphisms of the human mu opioid receptor gene, and the susceptibility of a subject to addictive diseases, such as heroin addiction, cocaine addiction, or alcohol addiction, to name only a few.

What is also needed are diagnostic methods to determine a subject's increased or decreased susceptibility to addictive diseases. With the results of such methods, targeted prevention methods, early therapeutic intervention, and improved chronic treatment to opioid addiction can be developed. Physicians armed with the results of such diagnostic methods can determine whether administration to a subject of opioid analgesics is appropriate or whether non-opioid derived analgesics should be administered to the subject. Also, appropriate choice and type of analgesic can be made in treating a subject's pain.

What is also need are methods of determining a subject's susceptibility to pain and responsibility to analgesics, and using that information when prescribing analgesics to the subject.

What is also needed is an ability to determine the binding affinity of the mu opioid receptor to endogenous opioids, such as β-endorphin, and the effect of this binding activity on the neuroendocrine system.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

There is provided, in accordance with the present invention, heretofore unknown polymorphisms of the human mu opioid receptor gene, and their use in mapping the locus of the human mu opioid receptor gene, determining susceptibility to addictive diseases, determining susceptibility to pain, and determining a therapeutically effective amount of pain reliever to administer to a subject suffering from pain, diagnosing a disease or disorder in a subject that is related to a physiological function regulated by the HPA or HPG axes of the neuroendocrine system, and selecting an appropriate therapeutic agent and a therapeutically effective amount of such an agent to administer to a subject suffering from a disease or disorder related to a physiological function regulated by the HPA or HPG.

Hence, the present invention extends to heretofore unknown polymorphisms of the human mu opioid receptor gene that can serve as genetic markers to map the locus of the human mu opioid receptor gene.

The present invention further extends to DNA sequences of heretofore unknown isolated nucleic acid molecules which encode human mu opioid receptors, wherein the DNA sequences include any combination of presently known polymorphisms and polymorphisms of the human mu opioid receptors discovered by Applicants.

The present invention further extends to the characterization of the binding properties of heretofore unknown human mu opioid receptors produced from the expression of isolated nucleic acid molecules comprising DNA sequences with such heretofore unknown polymorphisms of the human mu opioid receptor gene, or combinations of unknown polymorphisms and known polymorphisms.

The present invention further extends to Applicants' discovery that polymorphisms in an allele comprising a DNA sequence of SEQ ID NO:1, such as T67C, T124A, C153T, G174A, and

the addition of GGC (a glycine codon) following position 187, hereinafter abbreviated as 187INS:GGC, which are described in further detail *infra*, are present in the population. The T67C polymorphism changes serine 23 to a proline (hereinafter abbreviated Ser23Pro), the T124A polymorphism changes serine 42 to a threonine (hereinafter abbreviated Ser42Thr), and the 187INS:GGC adds a glycine residue following glycine 63. The C153T and G174A are silent mutations in the coding region of the mu opioid receptor gene.

The present invention further extends to diagnostic methods to determine a subject's increased or decreased susceptibility to addictive diseases. With the results of such methods, targeted prevention methods, early therapeutic intervention, and improved chronic treatment to opioid addiction are set forth herein and encompassed by the present invention. In addition, attending medical professionals armed with the results of such diagnostic methods can determine whether administration of opioid analgesics is appropriate or whether non-opioid derived analgesics should be administered to the subject. Furthermore, appropriate choice and type of analgesic to treat a subject's pain can be made. Such determination may be made by identification of any individual or any combination of the above-mentioned polymorphisms, using such non-limiting methods as DNA sequencing, differential hybridization to biological chip arrays such as an oligonucleotide gelpad microchip, or single nucleotide extension (SNE) on chip arrays such as on oligonucleotide gelpad microchips.

Also, the present invention extends to methods of determining a subject's increased or decreased susceptibility to pain and response to analgesics, and the use of the information in prescribing analgesics to the subject.

In addition, the present invention extends to methods of diagnosing a disease or disorder in a subject, wherein the disease or disorder is related to a physiological function regulated by the HPA or HPG axes of the neuroendocrine system. Examples of such physiological functions include reproductive or sexual functions, gastrointestinal motility, immune response, and ability to withstand stress.

Broadly the present invention extends to an isolated variant allele of a human mu opioid

receptor gene which can serve as a genetic marker, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

Furthermore, the present invention extends to an isolated variant allele of a human mu opioid receptor gene as set forth above, which is detectably labeled. Numerous detectable labels have applications in the present invention, such as radioactive elements, chemicals which fluoresces, or enzymes, to name only a few.

The present invention further extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of the human mu opioid receptor gene, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

Moreover, the present invention extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of the human mu opioid receptor gene, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof, wherein the isolated nucleic acid molecule is detectably labeled. Examples of detectable labels that have applications in this embodiment of the present invention are described above.

In addition, the present invention extends to an isolated variant allele of a human mu opioid receptor gene, wherein the predominant or "most common" allele of the human mu opioid

receptor gene encodes a human mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2, and the variant allele of the human mu opioid receptor gene encodes a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or the combination thereof.

Furthermore, the present invention extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene of the present invention, wherein the isolated nucleic acid molecule encodes a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or the combination thereof.

Naturally, the present invention extends to a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or the combination thereof.

Furthermore, the present invention extends to an antibody having as immunogen a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro, Ser42Thr, or the addition of a Gly residue following Gly63, or both. Such an antibody can be a polyclonal antibody, a monoclonal antibody, or a chimeric antibody. Moreover, an antibody of the present invention can be detectably labeled. Examples of detectable labels which have applications in this embodiment comprises a radioactive element, a chemical which fluoresces, or an enzyme, to name only a few.

In addition, the present invention extends to cloning vectors that can be used to clone copies of a variant alleles of a human mu opioid receptor gene of the present invention. For example, the present invention extends to a cloning vector comprising an isolated variant allele of a human mu opioid receptor gene and an origin of replication, wherein the predominant or "most

common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

In another embodiment, the present invention extends to a cloning vector comprising an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene, and an origin of replication, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

Numerous cloning vectors have applications in the present invention. For example, a cloning vector having applications in the present invention includes *E. coli*, bacteriophages such as lambda derivatives, plasmids such as pBR322 derivatives, and pUC plasmid derivatives such as pGEX vectors or pmal-c or pFLAG, to name only a few.

Naturally, the present invention extends to expression vectors comprising an isolated variant allele a human mu opioid receptor gene operatively associated with a promoter, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

Furthermore, the present invention extends to an expression vector comprising an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele a human mu opioid receptor gene, wherein the isolated nucleic acid molecule is operatively associated with a promoter. As set forth above, the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and

a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

Numerous promoters have applications in an expression vector of the present invention, including but not limited to immediate early promoters of hCMV, early promoters of SV40, early promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late promoters of SV40, late promoters of adenovirus, late promoters of vaccinia, late promoters of polyoma, the *lac* the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of phage lambda, control regions of fd coat protein, 3-phosphoglycerate kinase promoter, acid phosphatase promoter, or promoters of yeast α mating factor, to name only a few.

In addition, the present invention extends to a unicellular host transformed or transfected with an expression vector of the present invention. Examples of hosts which can be transformed or transfected with an expression vector of the present invention, and have applications in the present invention, include, but are not limited to, *E. coli*, Pseudomonas, Bacillus, Streptomyces, yeast, CHO, R1.1, B-W, L-M, COS1, COS7, BSC1, BSC40, BMT10 or Sf9 cells.

Naturally, the present invention extends to a method of producing a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro, Ser42Thr, or the addition of a Gly residue following Gly63, or the combination thereof. An example of such a method comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising an isolated variant allele a human mu opioid receptor gene, wherein the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A or 187INS:GGC, or the combination thereof, operatively associated with a promoter. The transformed or transfected unicellular host is then cultured under conditions that provide for

expression of the variant allele of the human mu opioid receptor gene. The variant human mu opioid receptor produced from such induced expression is then recovered from the unicellular host.

Another example comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising an isolated nucleic acid molecule operatively associated with a promoter, wherein the isolated nucleic acid molecule is selectively hybridizable to a variant allele a human mu opioid receptor gene, and the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and the variant allele comprises a DNA sequence having at least one variation in SEQ ID NO:1, wherein the at least one variation comprises T67C, T124A or 187INS:GGC, or the combination thereof. The transformed or transfected unicellular host is then cultured under conditions that provide for expression of the variant allele of the human mu opioid receptor gene. The variant human opioid receptor produced from such induced expression is then recovered from the unicellular host.

The invention further extends to altered expression of the mu opioid gene product, and means for detecting the altered expression, as a consequence of the presence of the silent mutations C153T or G174A, or the combination of either or both of the foregoing with any of the other polymorphisms hereindescribed.

Furthermore, the present invention extends to an isolated variant allele of a human mu opioid receptor gene, wherein the predominant or "most common" allele of the human mu opioid receptor gene comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one of the variations is T67C, T124A, C153T, G174A, or 187INS:GGC. The other variation may be any at least one of those described herein or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

The present invention further extends to an isolated variant allele of a human mu opioid receptor gone comprising a DNA sequence having at least two variations in SEQ ID NO:1, as

stated above, which is detectably labeled. Examples of detectable labels having applications in this embodiment include, but are not limited to, a radioactive element, a chemical which fluoresces, or an enzyme.

The present invention further extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene, wherein the predominant or "most common" allele of the human mu opioid receptor gene comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one of the variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be any at least one of those described herein or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Naturally, the present invention extends to a detectably labeled isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor comprising a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one of the variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be at least one of those described herein or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Examples of detectable labels having applications in this embodiment of the invention include, but are not limited to, a radioactive element, a chemical which fluoresces, or an enzyme.

Furthermore, the present invention extends to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, as set forth above, wherein the predominant or "most common" allele of a human mu opioid receptor gene encodes a human mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2, and a variant allele of the present invention encodes a human mu opioid receptor comprising an amino acid having at least two variations in SEQ ID NO:2, wherein the variations comprise Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or both, or at least one of the foregoing or at least one known in the art, such as but not limited to

Asn40Asp, Ala6Val, or Arg260His.

The present invention further extends to an isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, wherein the variations comprise T67C, T124A, C153T, G174A, or 187INS:GGC, wherein at least one of the variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be any at least one of those described herein or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A, such that the isolated nucleic acid molecule encodes a variant human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise at least one of Ser23Pro or conserved variants thereof, Ser42Thr or conserved variants thereof or the addition of a Gly residue following Gly63 or conserved variants thereof, and the other being at least the other of the foregoing or at least one variant known in the art, such as but not limited to Asn40Asp, Ala6Val, or Arg260His.

Naturally, the present invention extends to a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variations comprise:

Ser23Pro or conserved variants thereof;
Ser42Thr or conserved variants thereof;
or the addition of a Gly residue following Gly63 or conserved variants thereof.

Moreover, the present invention extends to an antibody having as an immunogen a human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise at least one of Ser23Pro or conserved variants thereof, Ser42Thr or conserved variants thereof or the addition of a Gly residue following Gly63 or conserved variants thereof, and the at least one other being at least one of the other of the foregoing or at least one variant known in the art, such as but not limited to Asn40Asp, Ala6Val, or Arg260His.

An antibody of the present invention can be a polyclonal antibody, a monoclonal antibody, or a chimeric antibody. Moreover, an antibody of the present invention can be detectably labeled. Examples of detectable labels having applications in an antibody of the present invention include, but are not limited to, a radioactive element, a chemical which fluoresces, or an enzyme.

Furthermore, the present invention extends to a cloning vector comprising an isolated variant allele of a human mu opioid receptor gene and an origin of replication, wherein the predominant or "most common" allele of the human mu opioid receptor gene present in the population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C, T124A; C153T; G174A or 187INS:GGC, and the at least one other being one other of the foregoing or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

In addition, the present invention extends to a cloning vector comprising an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor and an origin of replication, wherein the variant allele comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C, T124A; C153T; G174A or 187INS:GGC, and the at least one other being one other of the foregoing or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A; and an origin of replication.

Numerous cloning vectors have applications in this embodiment of the present invention. Examples of such vectors include, but are not limited to, *E. coli*, bacteriophages, such as lambda derivatives, plasmids such as pBR322 derivatives, and pUC plasmid derivatives such as pGEX vectors or pmal-c or pFLAG, to name only a few.

Naturally, the present invention extends to an expression vector comprising an isolated variant allele of a human mu opioid receptor gene operatively associated with a promoter, wherein such an isolated variant allele comprises a DNA sequence having at least two variations in SEQ

ID NO:1, wherein at least one the variations is T67C; T124A; C153T; G174A or 187INS:GGC, and the at least one other being one other of the foregoing or at least one variant known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

In addition, the present invention extends to an expression vector comprising an isolated nucleic acid molecule operatively associated with a promoter, wherein the isolated nucleic acid molecule is selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C; T124A; C153T; G174A or 187INS:GGC, and the at least one other variation being one other of the foregoing or at least one variant known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Numerous promoters are available and have applications in an expression vector of the present invention. Examples of promoters having applications include, but are not limited to immediate early promoters of hCMV, early promoters of SV40, early promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late promoters of SV40, late promoters of adenovirus, late promoters of vaccinia, late promoters of polyoma, the *lac* the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of phage lambda, control regions of fd coat protein, 3-phosphoglycerate kinase promoter, acid phosphatase promoter, or promoters of yeast α mating factor, to name only a few.

Naturally, the present invention extends to a unicellular host transformed or transfected with an expression vector of the present invention. Examples of unicellular hosts having applications in an embodiment of the present invention include, but are not limited to, *E. coli*, Pseudonomas, Bacillus, Streptomyces, yeast, WHO, R1.1, B-W, L-M, COS1, COS7, BSC1, BSC40, BMT10 or Sf9 cells.

In another embodiment, the present invention extends to a method for producing a human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise at least one of Ser23Pro or conserved variants thereof, Ser42Thr or conserved variants thereof or the addition of a Gly residue following Gly63, or

conserved variants thereof; and the at least one other being the other of the foregoing or at least one variant known in the art, such as but not limited to Asn40Asp, Ala6Val, or Arg260His.

More specifically, an example of a method for producing such a human mu opioid receptor comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising an isolated variant allele of a human mu opioid receptor gene operatively associated with a promoter, wherein the variant allele comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C; T124A; C153T; G174A or 187INS:GGC, and the at least one other variation being one other of the foregoing or at least one variant known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A; under conditions that provide for expression of the isolated variant allele of a human mu opioid receptor gene. After expression, a variant human mu opioid receptor is recovered from the unicellular host.

In another example, a method for producing a human mu opioid receptor of the present invention comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising an isolated nucleic acid molecule operatively associated with a promoter, wherein the isolated nucleic acid molecule is selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C; T124A; C153T; G174A or 187INS:GGC, and the at least one other variation being one other of the foregoing or at least one variant known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A, under conditions that provide for expression of the isolated nucleic acid molecule. The variant human mu opioid receptor produced from the expression is then recovered from the unicellular host.

The present invention also embraces functional variants of the mu opioid receptor as a consequence of the presence of at least one of the polymorphisms described herein, either as the only polymorphism as compared to the wild-type gene or in combination with any number of other polymorphisms, including the others described herein or those known in the art. The

invention is further directed to methods for detecting altered gene product structure, activity or function, said altered structure, activity or function resulting from the presence of at least one of the polymorphisms described herein.

Accordingly, the present invention extends to a method for determining a susceptibility in a subject to at least one addictive disease, comprising the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether the first allele comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C; T124A; or 187INS:GGC.

The present of at least one of these variations in the human mu opioid receptor gene of the first allele is expected to be indicative of the subject's susceptibility to at least one addictive disease relative to the susceptibility of a standard to at least one addictive disease, wherein the standard comprises a first allele comprising a human mu opioid receptor gene having a DNA sequence of SEQ ID NO:1.

Another embodiment of the method for determining a susceptibility in the subject to at least one addictive disease, as described above, comprises the further step of determining whether the second allele of the bodily sample of the subject comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variations comprise T67C, T124A or 187INS:GGC.

The presence of at least one variation the second allele of the bodily sample is expected to be indicative of the subject's susceptibility to at least one addictive disease relative to a standard in which both alleles of a human mu opioid receptor gene comprise a DNA sequence of SEQ ID NO:1.

In another embodiment, the present invention extends to a method for determining a susceptibility to at least one addictive disease in a subject relative to susceptibility to at least one addictive disease in a standard, involving the detection of variations in the human mu

opioid receptor itself, and particularly, determining whether a variant human mu opioid receptor is present in a bodily sample from a subject. Such a method comprises the steps of removing a bodily sample comprising a human mu opioid receptor from the subject, and determining whether the human mu opioid receptor present in the sample is a variant human mu opioid receptor of the invention, wherein the variant human mu opioid receptor comprises an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the at least one variation comprises: Ser23Pro, Ser42Thr or conserved variants thereof; or the addition of a Gly residue following Gly63 or conserved variants thereof, the presence of at least one variation is expected to be indicative of the subject's susceptibility to at least one addictive disease relative to susceptibility to at least one addictive disease in a standard, wherein the human mu opioid receptor of the standard comprises an amino acid sequence of SEQ ID NO:2.

As explained above, at least one addictive disease includes, but is not limited to, opioid addiction, cocaine addiction or addiction to other psychostimulants, nicotine addiction, barbiturate or sedative hypnotic addiction, anxiolytic addiction, or alcohol addiction.

Furthermore, the present invention extends to a method for determining a susceptibility to pain in a subject relative to susceptibility to pain in a standard, comprising the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether the first allele comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC. The presence of at least one variation in the human mu opioid receptor gene of the first allele is expected to be indicative of a decreased or increased susceptibility to pain in the subject relative to susceptibility to pain in the standard, wherein the first allele of the standard comprises a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Moreover, a method for determining a susceptibility to pain in a subject may further comprise the step of determining whether the second allele comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC. The presence of the at least one variation

in the human mu opioid receptor gene of the second allele of the bodily sample from the subject is expected to be indicative of an increased or decreased susceptibility to pain in the subject relative to the susceptibility to pain in the standard, wherein the second allele in the standard comprises a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

In another embodiment, the present invention extends to a method for determining a susceptibility to pain in a subject relative to susceptibility to pain in a standard by examining a bodily sample taken from the subject for the presence of a variant human mu opioid receptor. Such a method comprises the steps of removing a bodily sample comprising a human mu opioid receptor from the subject, and determining whether the human mu opioid receptor present in the sample is a variant human mu opioid receptor of the invention, i.e., comprises an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variation comprises:

Ser23Pro or conserved variants thereof;

Ser42Thr or conserved variants thereof; or

addition of a Gly residue following Gly63 or conserved variants thereof,

such that the presence of at least one variation is expected to be indicative of the subject's susceptibility to pain relative to susceptibility to pain in the standard, wherein the human mu opioid receptor of the standard comprises an amino acid sequence of SEQ ID NO:2.

Once a susceptibility to pain in the subject has been determined, it is possible for attending medical professionals treating the subject to administer to an appropriate, or therapeutically effective amount of pain reliever in order to induce analgesia in the subject. Administration of such an amount is important to the subject because, should an inappropriate amount of pain reliever be administered, the subject may not experience analgesia, and may be exposed to potentially deleterious side effects of the pain reliever, such as induction of addiction to the pain reliever, brain damage, or death.

Consequently, the present invention extends to a method for determining a therapeutically effective amount of pain reliever to administer to a subject in order to induce analgesia in the subject relative to a therapeutically effective amount of the pain reliever to administer to a

standard in order to induce analgesia in the standard, wherein the method comprises determining a susceptibility to pain in the subject relative to susceptibility to pain in the standard. The susceptibility of pain in the subject is expected to be indicative of the therapeutically effective amount of the pain reliever to administer to the subject to induce analgesia in the subject relative to the amount of the pain reliever to administer to the standard to induce analgesia in the standard.

Hence, the present invention extends to a method for determining a therapeutically effective amount of pain reliever to administer to a subject in order to induce analgesia in the subject relative to a therapeutically effective amount of the pain reliever to administer to a standard in order to induce analgesia in the standard wherein the method comprises the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether the first allele comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the at least one variation comprises: T67C, T124A or 187INS:GGC. The presence of at least one variation in the human mu opioid receptor gene of the first allele from the bodily sample is expected to be indicative of the therapeutically effective amount of pain reliever to administer to the subject to induce analgesia in the subject relative to the therapeutically effective amount of pain reliever to administer to the standard to induce analgesia in the standard, wherein the standard comprises a first allele comprising a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Moreover, the present invention further extends to a method for determining a therapeutically effective amount of pain reliever to administer to a subject in order to induce analgesia in the subject relative to a therapeutically effective amount of pain reliever to administer to a standard to induce analgesia therein, further comprising the steps of removing a bodily sample comprising a first and second allele comprising a human mu opioid receptor gene from the subject, and determining whether the second allele of the bodily sample comprises a human mu opioid receptor gene comprising a DNA sequence comprising at least one variation in SEQ ID NO:1, wherein the at least one variation comprises: T67C, T124A or 187INS:GGC. The presence of at least one variation in the human mu opioid receptor gene of the first and/or

second allele of the bodily sample is expected to be indicative of the therapeutically effective amount of pain reliever to administer to the subject to induce analgesia therein relative to the amount of pain reliever to administer to a standard to induce analgesia therein, wherein the first and second alleles of the standard comprise a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

In another embodiment, the present invention extends to determining a therapeutically effective amount of pain reliever to administer to a subject in order to induce analgesia in the subject, by examining a bodily sample from a subject for the presence of a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2. More specifically, the present invention extends to a method for determining a therapeutically effective amount of pain reliever to administer to a subject in order to induce analgesia in the subject, relative to a therapeutically effective amount of pain reliever to administer to a standard in order to induce analgesia in the standard, comprising the steps of removing a bodily sample comprising a human mu opioid receptor from the subject, and determining whether the human mu opioid receptor present in the sample comprises an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variation comprises:

Ser23Pro or conserved variants thereof;

Ser42Thr or conserved variants thereof; or

addition of a Gly residue following Gly63 or conserved variants thereof,

such that the presence of at least one variation is expected to be indicative of the therapeutically effective amount of pain reliever to administer to the subject to induce analgesia therein relative to the therapeutically effective amount of pain reliever to administer to induce analgesia in the standard, wherein the human mu opioid receptor of the standard comprises an amino acid sequence of SEQ ID NO:2.

Examples of pain relievers having applications in this embodiment of the present invention include, but are not limited to, morphine, codeine, dihydromorphin, meperidine, methadone, fentanyl and its congeners, butorphenol, nalbuphine, LAAM, or propoxyphine, to name only a few.

Furthermore, the present invention extends to a method for determining a therapeutically effective amount of a therapeutic agent for treating at least one addictive disease to administer to a subject suffering from at least one addictive disease, relative to a therapeutically effective amount of the therapeutic agent to administer to a standard suffering from the at least one addictive disease. As a result, the dosage of therapeutic agent administered to an addict can be "tailored" to the addict's needs based upon the addict's genotype. An example of such a method comprises the steps of removing a bodily sample from the subject, wherein the bodily sample comprises a first and second allele of the human mu opioid receptor gene, and determining whether the first allele comprises a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC. The presence of the at least one variation in the human mu opioid receptor gene of the first allele in the bodily sample from the subject is related to the therapeutically effective amount of therapeutic agent to administer to the subject to treat the subject's at least one addictive disease, relative to the therapeutically effective amount of the therapeutic agent to administer to the standard suffering from the at least one addictive disease, wherein the first and second allele of the standard comprise a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Furthermore, a method for determining a therapeutically effective amount of therapeutic agent to administer to a subject suffering from at least one addictive disease may further comprise an additional step of determining whether the second allele of the bodily sample taken from the subject comprises a human mu opioid receptor gene comprises a DNA sequence having at least one variation in SEQ ID NO:1, wherein the at least one variation comprises: T67C, T124A or 187INS:GGC. Such a variation in the first and/or second allele of the bodily sample is expected to be indicative of the therapeutically effective amount of the therapeutic agent to administer to the subject to treat the at least one addictive disease of the subject relative to the therapeutically effective amount of the therapeutic agent to administer to the standard suffering from the at least one addictive disease.

In another embodiment, the present invention extends to determining a therapeutically effective amount of a therapeutic agent for treating at least one addictive disease to administer to a